

Allelopathic effect of larch leaching on ash growth in mixed forest

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Abstract: Allelopathic effect of larch (*Larix gmelini*) leaching on ash (*Fraxinus mandshurica*) growth was studied with sand culture test. It was understood that the ash growth was stimulated by the larch leaching. The chemical compositions of the larch and ash leaching were identified and determined by chemical and instrumental analysis. The results showed that in the larch and ash leaching there were fatty acids (including monocarboxylic, dicarboxylic and tricarboxylic acids), phenolic acids, tannin, aromatic alcohols, polyol, aminoacids and carbohydrate, etc. On the basis of the analytical results of chemical composition, with reference to the results of model compound test, it is considered that the citric acid in the larch leaching was the main stimulative allelochemical to the ash growth, and trihydroxybenzeneacetic acid was the subordinate stimulative allelochemical.

Key words: Larch; Ash; Allelopathy leaching; Mixed forest

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Introduction

Concerning the mechanism of the interaction between tree species in the larch (*Larix gmelini*)-ash (*Fraxinus mandshurica*) mixed forest, we have studied the allelopathic effects of the larch volatile matter, litter and root secretion on the growth of ash. The obtained results showed clearly that the larch volatile matter played an inhibitive part, and both the larch litter and root secretion played an obviously stimulating part on the ash growth (Wu 2000a, 2000b, 2000c). This paper makes a study of another allelopathic way (leaching) between larch and ash. The leaching is a matter, which is solubilised in water as the tree is rinsed by rain water. There are various leaching, for example, the leaching from tree, the leaching from stem and the leaching from crown, etc. In this paper, the leaching was from tree. The leaching should contained mainly water-soluble mater, also a small amount of less soluble matter. The various chemical components in the leaching can show the stimulative or inhibitive allelopathy effect on the ash growth. We have determined the allelopathy of the leaching by means of culture test, the chemical

composition of the leaching by means of chemical and instrumental method, and the main allelochemical with reference to the results of the model compound test.

Test methods

Preparation of the leaching

Put fresh branch (with leaf) and bark in a big funnel, and rinse them with distilled water for 12 h. Evaporate the leaching liquor with vacuum rotary evaporator, and then freeze dry. The dry matter was the leaching.

Analysis of aminoacid and tannin

Aminoacids are determined with Waters aminoacid autoanalyser. The color tests were used to the qualitative identification of tannin, including gelatin test, FeCl_3 test, HCl-vanillin test and HCl-HCHO test. The quantitative determination of tannin was by using the hide powder method (Sun 1992).

Analysis of organic acid and other compounds

The leaching was dissolved in methanol, and treated in the methanol solution with CH_2N_2 ether solution for methylation. After carrying out the methylation, the distilled water was added into this methanol-ether solution, the ether layer was separated and the water layer was extracted three times with ether. Combine the ether solution, and evaporate the ether to dry. Dissolve the residue with a little CH_2Cl_2 , and analyze the methylated products with GC and GC-MS methods.

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Evaporate the water solution extracted by ether to dry with vacuum rotary evaporator, and then treat the residue with trimethylchlorosilane and hexamethyldisalazane to carry out silanization. Evaporate the excessive reagent in high vacuum, and dissolve the trimethylsilyl ethers in heptane for injection into the gas chromatograph to carry out GC and GC-MS analysis.

Results and discussion

Culture test

In sand culture test, the net growth of experimental seedlings of ash raised 30% more than that of control group. This stimulative effect of larch leaching was also obtained in the imitate sample test land, only the growth rate (22%) was slightly lower. The results revealed there were stimulative allelochemicals in the larch leaching.

Aminoacids

Aminoacids in fresh bark of larch and ash were measured with Waters aminoacid autoanalyser. The results as shown in Table 1 revealed that the aminoacids in ash bark were more abundant in either variety or quantity than those of larch. From the measured results, we can conclude that the aminoacids are not the stimulative allelochemicals, therefore we do not make further study on them.

Tannin

The tannin in the leaching with color test was examined, and the results were listed in Table 2.

The positive results of larch leaching indicated that it contained tannin. Formations of dark green in FeCl_3 test and pink in HCl-vanillin test indicated that

the tannin in larch was condensed tannin, having phloroglucinol type A cycle. Determining tannin with hide powder method, the content of tannin in bark of fresh larch was 5.8%, but there was no tannin in larch leaf. According to the result of model test (Sun 1992), the effect of tannin in larch leaching on the ash growth is quite limited, although its content in larch bark is higher.

Table 1. Content of aminoacids in fresh bark $\mu\text{g/g}$

Aminoacid	Ash	Larch
Aspartic acid	0.12	0.14
Glutamate	0.35	0.31
Serine	0.31	0.40
Arginine + threonine	0.45	0.33
Picramic acid	0.11	
Valine	0.13	0.12
Methionine	0.04	
Cystine	0.01	
Isoleucine	0.11	0.09
Leucine	0.21	0.15
Phenylalanine	0.10	0.08
Lysine	0.11	0.10
Total	2.05	1.72

Table 2. Result of qualitative test of leaching

Leaching	Gelatin test	FeCl_3 test	HCl-HCHO test	HCl-V anillin
Larch	Precipitation	Dark green	Precipitation	Pink
Ash	-	-	-	-

Analytical results of methylated product

Table 3 and Table 4 showed the analytical results of methylated products of the leaching by GC and GC-MS methods.

Table 3. Analytical result of methylated product of larch leaching

No.	Compound	Molecular formula	Relative content (%)
1	Dimethyl succinate	$\text{C}_6\text{H}_{10}\text{O}_4$	1.01
2	Trimethyl citrate	$\text{C}_9\text{H}_{14}\text{O}_7$	9.15
3	Methyl 3,4-dimethoxybenzoate	$\text{C}_{10}\text{H}_{12}\text{O}_4$	2.80
4	Methyl 2,4,6-trimethoxybenzeneacetate	$\text{C}_{12}\text{H}_{16}\text{O}_5$	3.95
5	Methyl palmitate	$\text{C}_{17}\text{H}_{34}\text{O}_2$	7.10
6	Manool	$\text{C}_{20}\text{H}_{34}\text{O}$	16.70
7	Methyl linoleate	$\text{C}_{19}\text{H}_{34}\text{O}_2$	20.50
8	Methyl stearate	$\text{C}_{19}\text{H}_{38}\text{O}_2$	4.80
9	Manodiol	$\text{C}_{20}\text{H}_{34}\text{O}_2$	9.60
10	Methyl isopimarate	$\text{C}_{21}\text{H}_{32}\text{O}_2$	7.50
11	Methyl abietate	$\text{C}_{21}\text{H}_{32}\text{O}_2$	5.50
12	Unknown		11.38

Comparing Table 3 with Table 4, we can make out their similarity and difference in chemical composition.

(1)The contents of the volatile matters are too low

to be inspected.

(2)The leaching of larch and ash contains a large number of higher fatty acids.

(3) The leaching of larch and ash contains pheno-

lic acids and alcohols.

(4) The leaching of larch contains more water-soluble binary acid and ternary acid than that in ash leaching.

According to the reference dealing with the model compound test (Wu 2000c), the higher fatty acids were inhibitive to the ash growth, nonvolatile alcohols

were almost no effect, but benzeneacetic acid and water soluble binary and ternary acids exerted strong stimulative effect. Therefore, citric acid in the larch leaching is main stimulative allelochemical on the ash growth, and trihydrobenzeneacetic acid is the subordinate substance playing stimulative effect.

Table 4. Analytical result of methylated product of ash leaching

No.	Compound	Molecular formula	Relative content (%)
1	Methyl levulate	C ₆ H ₁₀ O ₃	2.30
2	Dimethyl succinate	C ₈ H ₁₀ O ₄	2.96
3	4-Methoxybenzoic alcohol	C ₈ H ₁₀ O ₂	6.07
4	4-Methoxybenzene ethanol	C ₉ H ₁₂ O ₂	13.95
5	3,4-Dimethoxybenzene ethanol	C ₁₀ H ₁₄ O ₃	25.64
6	Methyl 3,4-dimethoxybenzoate	C ₁₀ H ₁₂ O ₄	4.22
7	Methyl myristate	C ₁₅ H ₃₀ O ₂	3.43
8	Methyl palmitate	C ₁₇ H ₃₄ O ₂	7.47
9	Methyl stearate	C ₁₉ H ₃₈ O ₂	11.70
10	Unknown		22.26

Analytical results of silanized product

The GC and GC-MS analytical results of silanized products listed in Table 5 and Table 6 revealed that there were a large number of carbohydrate and polyol in the water solution extracted by ether.

As there was a variety of conformational isomer, the gas chromatogram of trimethylsilyl ether of carbohydrate is more complicated. In order to concision, the content of each carbohydrate is calculated by combining with various conformational isomers.

Table 5. Analytical result of the larch leaching with silanization

No.	Compound	Molecular formula	Relative content (%)
1	Glycerol	C ₃ H ₈ O ₃	1.11
2	Ribitol	C ₅ H ₁₂ O ₅	0.98
3	Monolinoleoylglycerol	C ₂₁ H ₃₈ O ₄	1.49
4	Arabonic acid	C ₅ H ₁₀ O ₆	1.02
5	Gluconic acid	C ₆ H ₁₂ O ₇	0.47
6	Butaldose	C ₄ H ₈ O ₄	0.63
7	Arabinose	C ₅ H ₁₀ O ₅	17.60
8	Xylose	C ₅ H ₁₀ O ₅	26.91
9	Ribose	C ₅ H ₁₀ O ₅	4.90
10	Fructose	C ₆ H ₁₂ O ₆	1.85
11	Mannose	C ₆ H ₁₂ O ₆	11.28
12	Galactose	C ₆ H ₁₂ O ₆	7.92
13	Glucose	C ₆ H ₁₂ O ₆	23.84

The results showed in Table 5 and Table 6 reveal that their chemical compositions were quite similar. In the reference, the carbohydrates and nonvolatile alcohols did not exert allelopathic effect on the ash growth (Wu 2000c). Although there are gluconic acid

and arabonic acid in the larch leaching, their contents are too small to play an obvious stimulative role. Therefore, we did not consider the matters in water solution extracted by ether to exert allelopathic effect on the ash growth.

Table 6. Analytical result of the ash leaching with silanization

No.	Compound	Molecular formula	Relative content (%)
1	2,3-Butanediol	C ₄ H ₁₀ O ₂	12.08
2	Glycerol	C ₃ H ₈ O ₃	1.96
3	Monolinoleoylglycerol	C ₂₁ H ₃₈ O ₄	6.89
4	Butaldose	C ₄ H ₈ O ₄	20.70
5	Arabinose	C ₅ H ₁₀ O ₅	1.13
6	Xylose	C ₅ H ₁₀ O ₅	4.75
7	Mannose	C ₆ H ₁₂ O ₆	7.15
8	Galactose	C ₆ H ₁₂ O ₆	11.20
9	Glucose	C ₆ H ₁₂ O ₆	34.14

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